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Previews

A Sip of GABA for the Cerebral Cortex

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Cortical and striatal interneurons are both generated within the ventral telencephalon, but their migratory journey takes them to very different destinations. Two articles in this issue (van den Berghe et al., 2013; McKinsey et al., 2013) add an important molecular component to our understanding of how, during development, interneurons reach the cerebral cortex.

The precise execution of the complex cognitive, sensory, and motor functions of the mammalian cerebral cortex is dependent on the correct integration of two main neuronal populations: GABAergic inhibitory interneurons (INs) and glutamatergic excitatory projection neurons (PNs). GABAergic INs play essential roles in controlling the response of PNs to afferent inputs, preventing excessive excitation and synchronizing the activity of PN subpopulations. Given their critical modulatory functions, it is not surprising that disruption of interneurons' fate specification, migration, and overall organization into balanced cortical microcircuitry can lead to severe pathological conditions (Levitt et al., 2004, Rossignol, 2011).

Different populations of both cortical and striatal INs have been fate mapped to neural progenitors in the medial ganglionic eminences (MGEs) (Marín and Rubenstein, 2001). However, the molecular mechanisms that control the acquisition of distinct identities by cortical and striatal INs and, in particular, how the process of fate specification relates to their migratory choices, are not completely elucidated and remain the subject of intense investigation.

Elegant prior studies have demonstrated that *Nkx2-1* is a key transcription factor governing fundamental aspects of fate specification and migration of MGE-derived interneurons. Indeed, constitutive loss of *Nkx2-1* causes the respecification of MGE (and preoptic area) progenitors, which acquire a more dorsal, lateral ganglionic eminence (LGE)-like identity. This results in a dramatic reduction of cortical and striatal interneuron populations, as well as projection neuron populations of the globus pallidus and other basal forebrain structures (Sussel et al., 1998). Within progenitors of MGE identity, *Nkx2-1* continues to be important, as the generation of both GABAergic and cholinergic interneuron classes is controlled downstream of it. In this context, *Nkx2-1* regulates the expression of either *Lhx6* or *Lhx8* to instruct the acquisition of GABAergic and cholinergic IN fates, respectively (Wonders and Anderson, 2006, Fragkouli et al., 2009).

More recent work has demonstrated, however, that *Nkx2-1* function is finely temporally and spatially tuned. Conditional removal of *Nkx2-1* from the MGE at different developmental time points (and different stage of differentiation) demonstrates that early in development (between E9.5 and E10.5) *Nkx2-1* is necessary to enable the generation of MGE-derived cortical and striatal interneurons rather than LGE derivatives. However, genetic ablation of *Nkx2-1* at later developmental stages (E12.5) does not change the total number of INs produced but it affects the generation of specific classes of cortical interneurons, favoring the formation of CR^+/VIP^+ cortical IN subtypes, which normally would have a caudal ganglionic eminence (CGE) origin (Butt et al., 2008). Finally, after initial fate specification, postmitotic levels of *Nkx2-1* expression continue to affect cortical interneuron development, as key guidance receptors (i.e., *Neuropilin 2*) that are necessary for cortical INs to be repelled from the striatum and migrate to the cortex are directly and negatively regulated by *Nkx2-1* (Nóbrega-Pereira et al., 2008).

Together, these prior studies illustrate the importance of finely controlled regulation of *Nkx2-1* expression for normal fate specification and migration of cortical GABAergic interneurons. However, the molecular mechanisms acting upstream of *Nkx2-1* and responsible for this level of control have been unknown. Two articles in this issue (McKinsey et al., 2013; van den Berghe et al., 2013) independently identify the transcription factor *Sip1* (also known as *Zfhx1b* and *Zeb2*) as a new regulator of cortical interneuron differentiation and dorsal migration acting upstream of *Nkx2-1*.

"Should I Stay or Should I Go"? Sip1 Control over Interneuron Migration to the Cerebral Cortex

Upon fate specification in the MGE, cortical interneurons must initiate dorsally directed migration to reach the cortex. Downregulation of *Nkx2-1* is necessary in this context to ensure repulsion from the developing striatum (Nóbrega-Pereira et al., 2008). Striatal interneurons, on the contrary, maintain high levels of *Nkx2-1* to be able to invade the striatum. How is tuning of *Nkx2-1* levels differentially achieved in these two interneuron populations? New work now provides evidence that the transcription factor *Sip1*, which was previously known for its non-cell-autonomous role in controlling neurogenesis of excitatory projection neurons in the cerebral cortex (Seuntjens et al., 2009), is necessary to modulate *Nkx2-1* levels within migrating cortical interneurons and to control their migration to the cortex (McKinsey et al., 2013, van den Berghe et al., 2013).

Within the ventral telencephalon, *Sip1* is expressed at progressively increased levels within postmitotic interneurons as they migrate toward the cortex, and its expression is maintained once they enter

the cortical plate. Using several *Cre* lines to conditionally delete *Sip1* in the ventral telencephalon, both groups find a striking reduction in the number of PV$^+$ and Sst$^+$ (MGE-derived) interneurons that reach the cortex in these mutants (Figure 1). This is accompanied by the ectopic accumulation of interneurons in the striatum (McKinsey et al., 2013) and other regions of the ventral telencephalon (van den Berghe et al., 2013). Interestingly, molecular analysis demonstrates that expression of *Nkx2-1* remains elevated in most tangentially migrating interneurons upon loss of *Sip1*, thus suggesting that in the absence of this transcription factor cortical interneurons are unable to downregulate *Nkx2-1*, a necessary requirement to reach the cortex (Nóbrega-Pereira et al., 2008).

Of note, while *Nkx2-1* is required for the generation of both GABAergic and cholinergic interneurons, loss of *Sip1* only affects GABAergic interneurons, controlling their decision to migrate to the cortex or locate in the striatum. Closely related *Nkx2-1+/Lhx8+* striatal cholinergic interneurons remain unaffected in the absence of *Sip1* (McKinsey et al., 2013). It is tempting to speculate that different populations of progenitors might exist in the MGE that specify interneurons of the GABAergic and cholinergic fate. Alternatively, *Sip1* might not regulate *Nkx2-1* directly, and rather require additional cofactors, which in turn would determine its specificity of function in GABAergic interneurons. Further work that elucidates at the single-cell level the temporal and spatial regulation of *Sip1* expression and elucidation of the molecular logic that governs expression of *Sip1* downstream targets should clarify these possibilities.

While the exact molecular mechanisms of *Sip1* action remain to be elucidated, McKinsey and colleagues demonstrate that *Sip1* itself is regulated downstream of *Dlx2*, which binds directly to two conserved enhancers necessary for *Sip1* expression. The data support a possible model by which *Dlx2* positively regulates expression of *Sip1*, which in turn negatively regulates (directly or indirectly) *Nkx2-1* levels to control migration of interneurons to the cortex (Figure 1A). However, other factors are at play in controlling this complex process. van den Berghe and collaborators find that at least part of *Sip1* control over interneuron migration to the cortex is mediated by the Netrin receptor *Unc5b*, which is repressed downstream of *Sip1* (Figure 1A). Indeed, at the functional level, downregulation of *Unc5b* alone can partially rescue the migratory defects displayed by cortical interneurons that lack *Sip1*. In the future it will be interesting to investigate how expression of *Unc5b* and other guidance receptors, most prominently *Neuropilin 2*, is coordinated to finely modulate the migration of interneurons to the cortex.

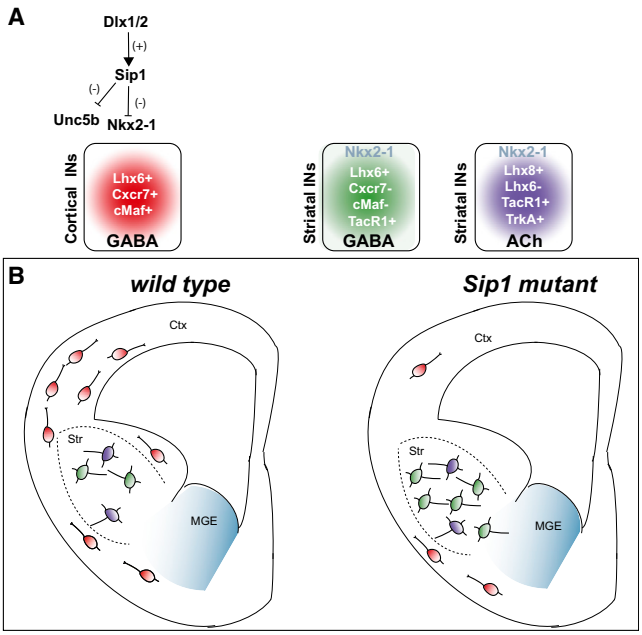

Figure 1. *Sip1* Is Required for Cortical Interneuron Differentiation and Migration to the Cerebral Cortex

(A) McKinsey et al. (2013) propose a model whereby in cortical interneurons *Dlx1/2* induce *Sip1* expression, which in turn negatively regulates *Nkx2-1* levels. van den Berghe et al. (2013) show that *Sip1* represses the expression of the guidance receptor *Unc5b*.

(B) In the absence of *Sip1*, up to 90% of PV$^+$ and Sst$^+$ cortical interneurons (MGE derived) fail to reach the cortex and stall in the ventral telencephalon. In addition, ectopically located interneurons downregulate markers of cortical interneurons and acquire molecular features of striatal GABAergic interneurons.

Beyond mechanistic explanations, the work defines *Sip1* as a novel transcription factor necessary to enable MGE-derived INs to populate the cortex.

"Dazed and Confused": Loss of Sip1 and the Identity Crisis for Cortical Interneurons

Conditional null mutant mice for *Sip1* survive for over three weeks postnatally, offering an opportunity to investigate the identity acquired by cortical interneurons misrouted to the subpallium in *Sip1* mutants. Both van den Berghe et al. (2013) and McKinsey et al. (2013) perform molecular profiling of neurons isolated from the subpallium of *Sip1* conditional mutants. While, perhaps unsurprisingly, the levels of differential expression reported for some genes differ slightly between the two studies (at least in part due to different methods of tissue collection), overall the data show that several transcripts normally present in cortical interneurons are downregulated in the mutants, while some genes preferentially expressed in striatal GABAergic interneurons appear upregulated. Downregulated genes include *Cxcr4*, *Gria1*, *Ets1*, *Cxcr7*, *Grik1*, *Cntnap4*, *Grip1*, *Chl1*, *Cacng2*, *Csdc2*, and *Scn1a* (van den Berghe et al., 2013), known to be enriched in, albeit not restricted to, developing cortical interneurons (Batista-Brito et al., 2008, Marsh et al., 2008, McKinsey et al., 2013). Similarly, *Cux2*, which preferentially labels cortical versus striatal interneurons, was reduced. In contrast, *Nkx2-1*, whose expression normally remains high in striatal interneurons but is downregulated in cortical interneurons, and NPY, which at E15.5 preferentially labels striatal interneurons, were upregulated in the mutant subpallium.

Despite the fact that a unique combinatorial code of molecules does not currently exist that can distinguish striatal and cortical GABAergic interneurons, this

molecular analysis shows not only that in the absence of *Sip1* cortical INs fail to migrate to the cortex and ectopically position in the striatum, but also that in this new environment they fail to acquire or maintain molecular features of cortical INs, gaining some traits of striatal GABAergic interneurons (Figure 1B). Consistent with this model, McKinsey and colleagues identify the Substance P receptor *TacR1* as being selectively expressed in striatal interneurons compared to cortical interneurons and show that its levels are increased in the *Sip1* mutant striatum. Notably, markers of cholinergic striatal interneurons (*Lhx8*, *Gbx2*, *Isl1*, and *TrkA*) and those of the globus pallidus (*Kcdt12*, *Gbx2*, and *Kcnmb4*) remain unchanged, suggesting that a switch of fate between GABAergic populations of cortical and striatal interneurons has occurred in the mutants.

The observed molecular changes could be due to a direct, cell-autonomous effect of *Sip1* loss, though one cannot exclude a non-cell-autonomous effect of the new, ectopic environment to which the "redirected" interneurons are now exposed. The fact that the small percentage of cortical neurons that manage to reach the cortex acquire expression of cortical IN features suggests that the environment of the striatum might also be playing a role in instructing this switch of fate. From this perspective, it would be interesting in the future to understand whether *Sip1* mutant interneurons for which migration has been rescued and which can thus reach the cortex, for example by downregulation of *Unc5b*, are still able to become bona fide cortical interneurons.

Finally, the changes in molecular identity observed in cortical interneurons upon loss of *Sip1* suggest that this transcription factor might have been important for the evolution of cortical inhibitory circuitry. Although mammalian brains are the only, among vertebrates, with a six-layer cerebral cortex, lower vertebrates might have already devised mechanisms to sort GABAergic interneurons fated to the subpallium from those destined to the pallium. Further comparative molecular analysis of different species may shed light on the evolutionary relevance of *Sip1* for the acquisition of cortical interneurons.

"With or without You": Reduction of Cortical Interneurons and Mowat-Wilson Syndrome

Many neurodevelopmental disorders and mental illnesses are caused by a malformed or malfunctioning cortical GABAergic circuit. This testifies to the importance of balanced cortical circuitry for high-level cortical function and justifies efforts to unravel the molecular mechanisms governing interneuron specification, positioning, and connectivity in the cortex. For some of these pathologies a genetic etiology is known while for others it remains elusive.

It is intriguing that mutations in the *Sip1* gene locus (2q22-q23) have been reported in patients suffering from a very complex syndrome known as Mowat-Wilson Syndrome (MWS). Indeed, since the first description of this disease in 1998, more than 100 *Sip1* mutations (including deletions) have been reported from patients all over the world (Mowat et al., 2003, Garavelli and Mainardi, 2007). This is interesting, because while patients with MWS show different levels of intellectual disability and motor impairment, seizures and abnormal EEGs have been reported in 90% of cases and thus represent a prominent clinical sign of this syndrome. In light of the role now reported for *Sip1* in controlling cortical interneuron differentiation and migration to the cortex, clinical symptoms of these patients could be explained by a decrease of two main populations of MGE-derived cortical interneurons, PV+ and Sst+.

Sip1 is known to also work non-cell-autonomously to control the rate of excitatory neuron birth in the cortex (Seuntjens et al., 2009), and a role for this protein as a regulator of oligodendrocyte differentiation and myelination has also been recently reported (Weng et al., 2012). Even with the caveat that disturbed excitatory projection neuron development could result in an abnormal cortical inhibitory circuit (Hevner et al., 2004, Lodato et al., 2011) and that defective myelination has been associated to epileptic phenotypes in mice, the new work on the role of *Sip1* over development of cortical interneurons centrally contributes to the understanding of MWS pathology and etiology and informs approaches for future therapeutic intervention.

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